Relationships between Environmental Organochlorine Contaminant Residues, Plasma Corticosterone Concentrations, and Intermediary Metabolic Enzyme Activities in Great Lakes Herring Gull Embryos

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Experiments were conducted to survey and detect differences in plasma corticosterone concentrations and intermediary metabolic enzyme activities in herring gull (*Larus argentatus*) embryos environmentally exposed to organochlorine contaminants *in ovo*. Unincubated fertile herring gull eggs were collected from an Atlantic coast control site and various Great Lakes sites in 1997 and artificially incubated in the laboratory. Liver and/or kidney tissues from approximately half of the late-stage embryos were analyzed for the activities of various intermediary metabolic enzymes known to be regulated, at least in part, by corticosteroids. Basal plasma corticosterone concentrations were determined for the remaining embryos. Yolk sacs were collected from each embryo and a subset was analyzed for organochlorine contaminants. Regression analysis of individual yolk sac organochlorine residue concentrations, or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs), with individual basal plasma corticosterone concentrations indicated statistically significant inverse relationships for polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDDs/PCDFs), total polychlorinated biphenyls (PCBs), non-*ortho* PCBs, and TEQs. Similarly, inverse relationships were observed for the activities of two intermediary metabolic enzymes (phosphoenolpyruvate carboxykinase and malic enzyme) when regressed against PCDDs/PCDFs. Overall, these data suggest that current levels of organochlorine contamination may be affecting the hypothalamo–pituitary–adrenal axis and associated intermediary metabolic pathways in environmentally exposed herring gull embryos in the Great Lakes. *Key words*: avian, corticosterone, endocrine disruption, environment, herring gull, metabolism, organochlorine. *Environ Health Perspect* 107:179–186 (1998). [Online 26 January 1999]
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Over the past 30 years a number of incidences of endocrine disruption in wildlife exposed to industrial chemicals have been reported. These effects have been observed in species as diverse as gastropods, fish, alligators, birds, seals, and other mammals (*1–10*). The natures of the endocrine lesions are equally diverse. Reproductive problems and behavioral abnormalities related to reproduction are among the most commonly reported endocrine-related effects of contaminant exposure. Depending on the species and contaminant, these effects have been documented as development of male secondary sex characteristics in females (*1*), abnormal steroid hormone concentrations (*2–5*), developmental abnormalities of gonads (*4,5*), and feminization of embryonic reproductive tracts (*6,7*). In addition, altered histology of the thyroid and adrenal glands have been reported in exposed wildlife (*8–10*).

Other effects of environmental contaminants, which are not as readily associated with disruption of endocrine pathways, have also been noted. Embryonic mortality and deformities, failure to thrive (wasting), and altered reproductive parameters measured as hatching success, number of young fledged, and length of egg incubation have been observed in various species of colonial fish-eating birds (*11–14*). Although the incidences of these biological end points are highly variable from site to site, a combination of chemical residue data and epidemiologic criteria support an important etiologic role for coplanar polychlorinated biphenyls (PCBs) and other dioxinlike organochlorines in some locations and species.

The hypothalamo–pituitary–adrenal (HPA) axis is an important system that regulates and integrates many physiologic functions in response to environmental stressors. Activation of the HPA axis during a stress response results in the sequential release of various hormones. Corticotropin-releasing factor, released from the hypothalamus, acts on the anterior pituitary to produce adrenocorticotropic hormone (ACTH). Subsequently, ACTH moves via the blood to stimulate secretion of corticosteroids from the adrenal gland. HPA axis activation, resulting in corticosteroid production, initiates several important physiologic changes in birds, including effects on intermediary metabolism, growth, immune function and inflammatory responses, and, possibly, enzymatic detoxification and microsomal oxidative metabolism pathways. Although chronic activation is detrimental to reproductive success and survival, short-term activation of this system enables birds to respond and adapt to stressors (*15*).

Based on a literature survey, Ribelin (*16*) reported that, of all the endocrine tissues, chemically induced morphologic lesions most frequently occurred in the adrenal gland. The high susceptibility of the adrenal gland to exogenous chemicals is thought to be due primarily to the high lipid content of the adrenal cortex. Because most xenobiotics that enter the body are hydrophobic, the lipid-rich adrenal cortex is susceptible to their deposition and accumulation (*17*). In addition, the adrenal gland has the potential to bioactivate exogenous compounds that are deposited there. It has recently been established that adrenal monooxygenases (P450 family) not only participate in steroid metabolism, but also have the capacity to metabolize xenobiotics, in some cases producing toxic metabolites (*17*).

Of particular interest and relevance to this paper are studies describing the reduced abilities of birds to cope with a secondary stress (cold temperatures) after exposure to the primary stressor, petroleum (*18,19*). Thus, it is conceivable that other environmental contaminants could similarly interfere with the HPA axis to increase the susceptibility of birds to nonanthropogenic environmental stressors. Avian embryos are well suited for the study of adrenal or HPA axis regulation because there is no interfering connection of the embryo to a maternal organism and because late-stage embryos of precocial avian species have a sufficiently mature HPA axis for studying its reactivity towards stressors *in ovo*, thus avoiding the influence of many complicating external environmental factors. To our knowledge, no prior avian HPA axis studies have considered the potential toxicologic effects of

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complex mixtures of environmental organochlorine contaminants. However, studies by Hontela et al. (*20–22*) and Gendron and colleagues (*23*) [reviewed by Hontela (*24*)] that characterized adrenal and HPA axis function in two species of wild freshwater fish and an amphibian (mud puppy) strongly indicated that HPA axis-related end points are altered after exposure of these animals to complex mixtures of contaminants. These data further support the idea that HPA axis-related end points are useful indicators of contaminant exposure and potential toxicity in wild animals.

In the present study, experiments were conducted to survey and detect differences in HPA axis-related end points in herring gull embryos environmentally exposed to various concentrations and mixtures of organochlorine contaminants *in ovo*. The data obtained were subjected to statistical analyses to test the hypotheses that exposure of embryonic herring gulls to environmental organochlorine contaminants influences plasma corticosterone concentrations and also alters the activities of intermediary metabolic enzymes that are regulated, at least in part, by corticosteroids.

Materials and Methods

Egg Collection and Incubation

During May and June 1997, unincubated fertile herring gull eggs were collected from Kent Island, New Brunswick, Canada (near Grand Manan Island in the Bay of Fundy; control site); Middle Island (western basin of Lake Erie); Snake Island (eastern basin of Lake Ontario); Hamilton Harbor (western terminus of Lake Ontario); and Papoose Island (near Thunder Bay, Ontario, in Lake Superior). The eggs were brought to the University of Ottawa (Ottawa, Ontario, Canada) or the National Wildlife Research Centre (Hull, Quebec, Canada), where they were artificially incubated for 24 or 26 days at 37.5°C and 60% relative humidity in a Curfew model RX250 incubator (Althorne, Essex, England) with automatic turning. The normal incubation period for herring gull eggs is 28 days. All experiments were approved and conducted in compliance with the Canadian Council on Animal Care.

Plasma Corticosterone

Blood from individual 24-day-old herring gull embryos from each site was collected and analyzed for corticosterone based on previously published methods (*25,26*). Briefly, a large blood vessel associated with the shell membrane was located and marked by candling the eggs on day 23 of incubation. A window was cut through the shell of each egg (but not the shell membrane) using a dental drill (Buffalo Dental Manufacturing Company, Inc., Syosset, NY) equipped with a small cut-off wheel (Dremel no. 409, Racine, WI) to allow access to the vessel. The window was covered with removable tape and all eggs were returned to the incubator without turning. On day 24 of incubation, the tape was removed from each egg and the membrane was allowed to dry. A drop of mineral oil was applied to the membrane to make it transparent and the selected vessel was cannulated using a 27G needle fitted to 0.30 mm i.d. silastic tubing (Dow Corning, Midland, MI). This tubing was attached to a three-way stopcock fitted with a heparinized disposable tuberculin syringe. A single blood sample (100 µl) was taken for basal corticosterone analysis. All embryos were sacrificed by decapitation and the yolk sacs were frozen for organochlorine residue analysis. Plasma was separated by centrifuging the blood samples, frozen in liquid nitrogen and stored at -80°C until analyzed for corticosterone, using a commercially available ^{125}I radioimmunoassay kit (Immunchem Double Antibody, ICN, Costa Mesa, CA). Intra- and interassay coefficients of variation were 4.4–10.3% and 6.5–7.2%, respectively. Cross-reactivity was 0.35% for desoxycorticosterone, 0.1% for testosterone, and < 0.05% for all other steroids tested. Recovery of exogenous corticosterone added to duck embryo or herring gull plasma samples was > 98% and the sensitivity of the assay was at least 25 ng/ml. Plasma dilutions of 1:20 were determined to be optimal for herring gull embryos.

Tissue Preparation

On day 26 of incubation, herring gull embryos from each site were sacrificed by decapitation. The liver was immediately removed, rinsed, and homogenized in 3 ml of Tris-buffered 0.25 M sucrose (5 mM Tris, pH 7.4) per gram of tissue. Cytosol fractions were prepared by differential centrifugation. Briefly, the homogenate was centrifuged at 1,000*g* for 10 min at 4°C (model 5402, Eppendorf Scientific, Inc., Westbury, NY) and the resulting supernatant was centrifuged at 10,000*g* for 10 min at 4°C [model J2-21M/E, Beckman Instruments (Canada) Inc., Mississauga, Ontario]. The resulting supernatant was filtered through a 1-µm syringe filter (glass fiber Acrodisc, Gelman Sciences, Ann Arbor, MI). After a final centrifugation of the filtrate at 105,000*g* for 1 hr at 4°C [Optima TL, Beckman Instruments (Canada) Inc.], the supernatant (cytosol) was frozen in aliquots in liquid nitrogen. Cytosol samples were similarly prepared from the kidneys of each embryo. All samples were stored at -80°C until they were analyzed for protein concentration (BCA, Pierce, Rockford, IL) and enzyme activities. Pools of liver and kidney cytosol prepared from both domestic duck embryos and Kent Island herring gull embryos were used to optimize each assay prior to analysis of individual samples.

Intermediary Metabolic Enzymes

The activities of four intermediary metabolic enzymes were assayed. Previously published methods were optimized and modified to allow measurement of samples in 96-well plates (Corning Costar, Acton, MA) using an ultraviolet-vis microplate reader (Spectracount Microplate Photometer, Canberra-Packard Canada Ltd., Mississauga, Ontario, Canada). For each enzyme, optimization involved determination of optimal cytosol protein and substrate concentrations that provided a linear response for at least 10 min and absorbances that did not exceed the bounds of the reduced nicotamine adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH) standard curves. All concentrations given are based on final reaction volumes, and NADH or NADPH standards were included in quadruplicate on each plate. Assay plates for all four enzymes were scanned at 1-min intervals for approximately 10 min and the rate of the reaction for each sample (in quadruplicate) was determined by calculating the change in absorbance at 340 nm over time as compared to the sample blank (in quadruplicate). Enzyme activities are expressed as nmol NAD(H) or NADP(H) oxidized or reduced/min/mg protein.

Phosphoenolpyruvate carboxykinase (PEPCK). The method described by Petrescu et al. (*27*) was optimized for herring gull embryo liver and kidney cytosol samples. Kidney or liver cytosol (37.5 µl containing 0.15 mg total protein diluted with Tris-buffered 0.25 M sucrose as required) was added to 162.5 µl of a mixture of CO_2-saturated $NaHCO_3$ (20 mM), phosphoenolpyruvate (1 mM), NADH (0.1 mM), and malate dehydrogenase (0.5 U), all dissolved in Tris buffer (50 mM, pH 7.4). The plate was shaken on a microplate shaker for 30 sec. The reaction was initiated with 50 µl dGDP (2′-deoxyguanosine 5′-diphosphate, 0.2 mM) dissolved in Tris buffer for a final reaction volume of 250 µl. The absence of $NaHCO_3$ was used as a blank.

Malic enzyme (ME). The method described by Wise and Ball (*28*) was optimized for herring gull embryo liver cytosol samples. Liver cytosol (37.5 µl containing 0.15 mg total protein diluted with Tris-buffered 0.25 M sucrose as required) was added to 162.5 µl of a mixture of $MgCl_2$

(4.5 mM) and NADP (0.2 mM), each dissolved in Tris (50 mM, pH 7.4). The plate was shaken on a microplate shaker for 30 sec. The reaction was initiated with 50 µl sodium malate (2.5 mM) dissolved in Tris buffer. Malate was not added to the blank wells.

Alanine aminotransferase (ALAT). The method described by Segal and Matsuzawa (*29*) was optimized for herring gull embryo liver and kidney cytosol samples. Cytosol (50 µl containing 0.1 mg total protein for liver or 0.025 mg total protein for kidney diluted with Tris-buffered 0.25 M sucrose as required) was added to 150 µl of a mixture of α-ketoglutarate (4.1 mM), NADH (liver, 0.17 mM; kidney, 0.34 mM), and lactic dehydrogenase (0.42 U), all dissolved in phosphate buffer (0.1 M, pH 7.3). The plate was shaken on a microplate shaker for 30 sec. The reaction was initiated with 50 µl L-alanine (100 mM) dissolved in phosphate buffer. Alanine was not added to the blank wells.

Aspartate aminotransferase (ASPAT). The method described by Segal and Matsuzawa (*29*) for ALAT was adjusted and optimized for measuring ASPAT activities in herring gull embryo liver and kidney cytosol samples. Reaction conditions were identical to those described for ALAT except the total protein concentration of herring gull embryo liver and kidney cytosol was 0.01 mg protein/well. To start the reaction, 50 µl L-aspartate (100 mM) dissolved in phosphate buffer was added to all but the blank wells.

Chemical Residue Analysis

Twenty-six yolk sac samples, chosen randomly to represent each site and end point, were analyzed for polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), non-*ortho* substituted PCBs, organochlorine pesticides and other chlorinated hydrocarbons (CHCs), and total PCBs (sum of 59 congeners). The samples were analyzed by standard procedures at the National Wildlife Research Centre. These procedures are based on original publications by Norstrom and Simon (*30*) and Norstrom and Won (*31*). Based on a previous study in which the relative cytochrome P4501A (CYP1A)-inducing potencies (induction equivalency factors; IEFs) of specific organochlorine contaminants in primary hepatocyte cultures prepared from herring gull embryos were reported (*32*), yolk sac residue data were also expressed as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs). Expression of contaminant residue data in this manner allows estimation of the potential toxicity of complex environmental mixtures by taking into account the extreme range of potencies of specific isomers and congeners to induce CYP1A, their specific concentrations in a given sample, and interspecies differences in sensitivity to these chemicals (*33*).

Statistical Analysis

All chemical residue and biological measurement data were normally distributed (Kolmogorov–Smirnov test, $p < 0.05$). Contaminant levels and biological measurements were compared among sites with a one-way analysis of variance; significant differences were determined using Tukey's multiple comparison procedure. Relationships between contaminant levels and biological measurements were determined using coefficients of determination (R^2) obtained from simple linear regressions. Unless stated otherwise, a value of $p < 0.05$ was considered statistically significant in all analyses. All statistical analysis was conducted with Sigmastat statistical software (Jandel Scientific, Chicago, IL).

Results

Organochlorine Contaminant Levels

The organochlorine residue data for individual yolk sac samples were analyzed to determine means for each major class of contaminants for each site (Fig. 1). For all samples combined, the total PCB fraction accounted for >60% of the total residues, whereas the PCDD/PCDF fraction accounted for <1% of the total residues. Of the 59 congeners detected, PCB 153 was the dominant PCB found in the yolk sacs. Of the CHCs, *p,p'*-DDE accounted for

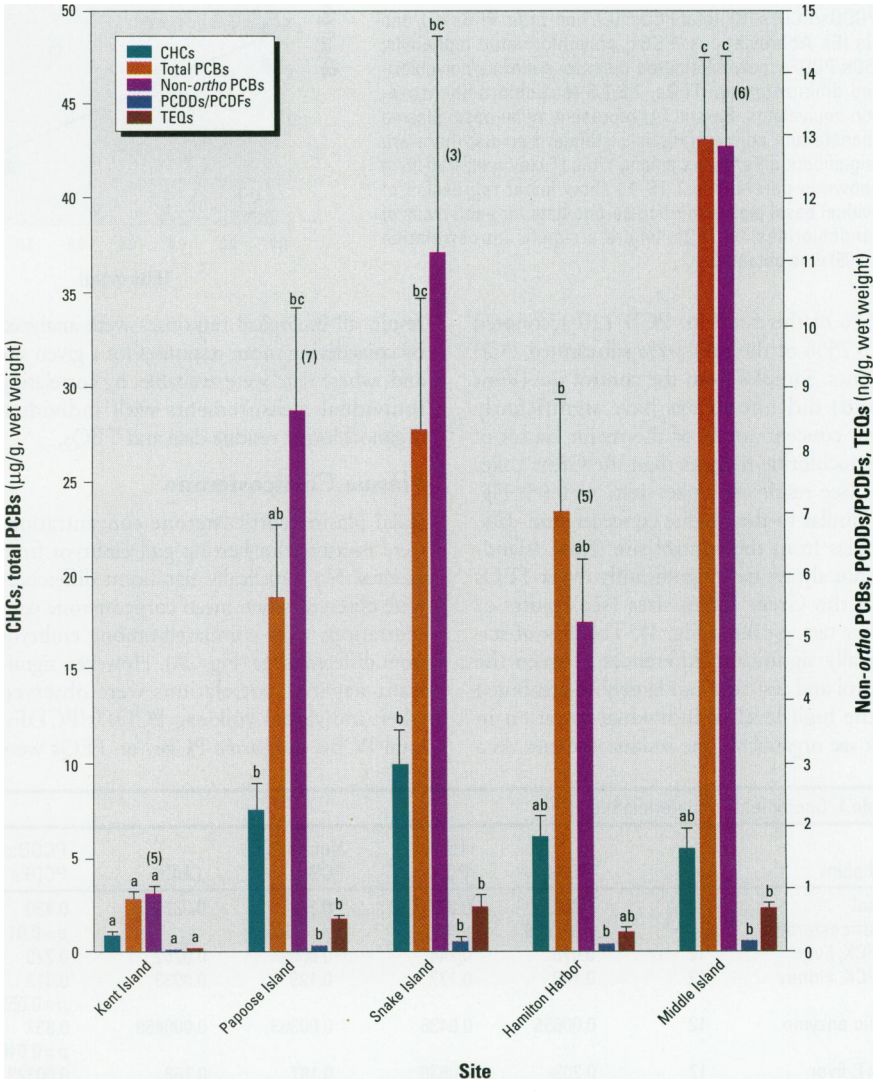

Figure 1. Mean organochlorine residues and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) in herring gull embryo yolk sacs. Abbreviations: CHCs, chlorinated hydrocarbons and organochlorine pesticides; PCBs, polychlorinated biphenyls; PCDDs/PCDFs, polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofuran. Bars for each class of organochlorines, or TEQs, identified with the same letter among sites are not significantly different from each other (Tukey test, $p < 0.05$; *n* is shown in parentheses); error bars indicate standard error.

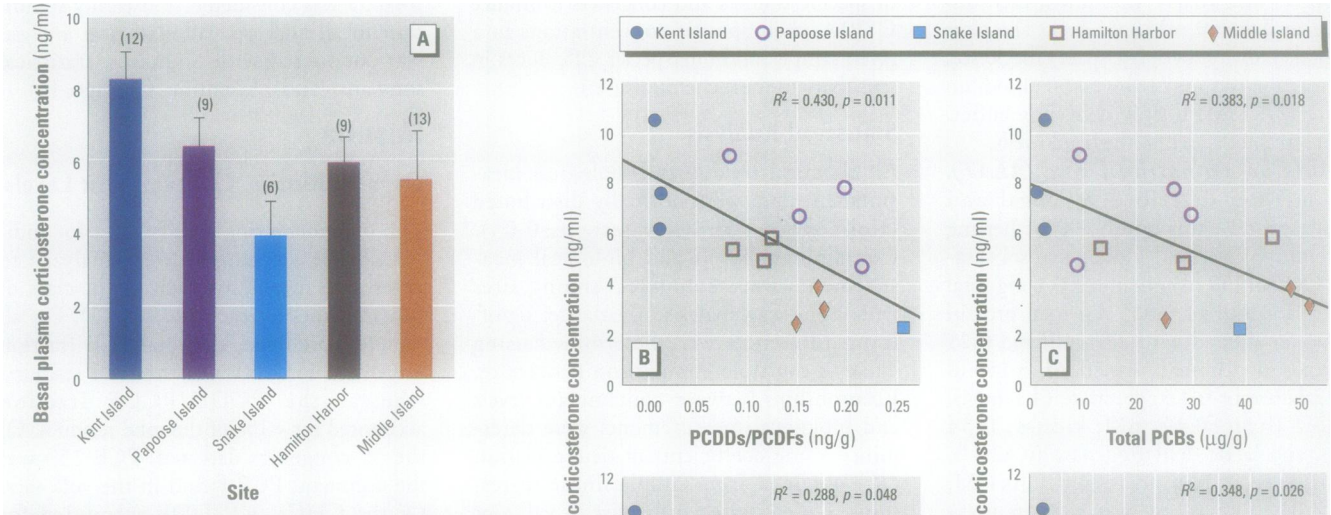

Figure 2. Basal plasma corticosterone concentrations in herring gull embryos by site (A) and by yolk-sac concentrations of PCDDs/PCDFs (B), total PCBs, (C), non-*ortho* PCBs (D), and TEQs (E). Abbreviations: PCBs, polychlorinated biphenyls; PCDDs/PCDFs, polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans; TEQs, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents. Bars in (A) represent mean basal plasma corticosterone concentrations ± standard errors; there are no significant differences among sites (Tukey test, $p>0.05$; *n* is shown in parentheses). (B–E) show linear regression of individual basal plasma corticosterone data for each class of organochlorines, or TEQs, where a significant correlation ($p<0.05$) was obtained.

>75% of this fraction. PCB 126 accounted for >75% of the non-*ortho* substituted PCB residues. Samples from the control site (Kent Island) did not always have significantly lower concentrations of the major classes of organochlorine residues than the Great Lakes sites (see results of Tukey test, $p<0.05$; Fig. 1). Similar to the residue concentration data, samples from the control site (Kent Island) did not always have significantly lower TEQs than the Great Lakes sites (see results of Tukey test, $p<0.05$; Fig. 1). The lack of statistically significant differences between the control and test sites can largely be attributed to the high level of individual variation in yolk sac organochlorine residue content. As a result, all biological responses were analyzed by considering mean responses for a given site and, where data were available, by correlating individual measurements with individual organochlorine residue data and TEQs.

Plasma Corticosterone

Basal plasma corticosterone concentrations were measured in herring gull embryos from all sites. No statistically significant differences were observed when mean corticosterone concentrations were compared among embryos from different sites (Fig. 2A). However, significant negative correlations were observed when individual yolk sac PCDDs/PCDFs, total PCBs, non-*ortho* PCBs, or TEQs were regressed against basal plasma corticosterone levels for the same individuals (Fig. 2B–E; Table 1). There was no significant correlation observed when yolk sac CHC concentrations were regressed against basal plasma corticosterone levels for individual embryos (Table 1; $p>0.05$).

Intermediary Metabolic Enzymes

The activities of four intermediary metabolic enzymes were measured in herring gull embryo liver or kidney cytosol, or both. Of the enzymes measured, only hepatic and renal PEPCK and hepatic ME activities tended to be lower for embryos collected at the Great Lakes sites than at the Atlantic site (Fig. 3A and 4A). However, in many cases, these activities were not statistically different from those measured for embryos collected at Kent Island (results of Tukey tests, for Fig. 3A and 4A).

Correlation coefficients for regression analysis of individual yolk sac organochlorine residue data, or TEQs, against PEPCK or ME activities are shown in Table 1. A significant negative correlation was obtained when individual yolk sac PCDD/PCDF concentrations were regressed against ME activities for the same individuals (Fig. 4B; Table 1). Regression of individual yolk sac PCDD/PCDF concentrations against kidney cytosolic PEPCK also indicates a possible relationship that approaches statistical significance ($p=0.059$, Fig. 3B; Table 1).

Table 1. Coefficients of determination (R^2)

End point	*n*	TEQs	Total PCBs	Non-*ortho* PCBs	CHCs	PCDDs/ PCDFs
Basal corticosterone	14	0.288 $p=0.048$	0.383 $p=0.018$	0.348 $p=0.026$	0.0851 —	0.430 $p=0.011$
PEPCK, liver	12	0.075	0.144	0.0692	0.0262	0.225
PEPCK, kidney	12	0.140	0.177	0.129	0.0933	0.312 $p=0.059$
Malic enzyme	12	0.00655	0.0436	0.00363	0.000459	0.357 $p=0.040$
ALAT, liver	12	0.203	0.0530	0.187	0.168	0.00323
ALAT, kidney	12	0.000134	0.00913	0.0000209	0.126	0.128
ASPAT, liver	12	0.0662	0.0234	0.0602	0.0543	0.0129
ASPAT, kidney	12	0.101	0.131	0.141	0.00123	0.0130

Individual biological measurements for each end point were linearly regressed against TEQs, or the concentrations of each of the four major classes of organochlorines, determined for the individual yolk sacs. The significance of the correlation was $p>0.05$ unless specifically indicated.

No statistically significant differences were observed among sites for either hepatic or renal ALAT or ASPAT activities (Fig. 5 and 6). Similarly, correlation coefficients obtained from regression of individual organochlorine residues, or TEQs, against the activities of ALAT or ASPAT for individual embryos, did not reveal any significant relationships ($p > 0.05$; Table 1).

Discussion

Studies were conducted to survey and detect differences in HPA axis-related end points in herring gull embryos environmentally exposed to various concentrations and mixtures of organochlorine contaminants *in ovo*. Although not apparent in site-to-site comparisons, regression analysis of individual yolk sac organochlorine residue concentrations against individual basal plasma corticosterone concentrations indicated a statistically significant inverse relationship for PCDDs/PCDFs, total PCBs, and non-*ortho* PCBs. A similar trend was observed when basal plasma corticosterone concentrations were regressed against TEQs calculated for the individual yolk sac residue data using IEFs obtained for herring gull embryo hepatocyte cultures. The activities of two intermediary metabolic enzymes regulated, at least in part, by corticosteroids—PEPCK and ME—were shown by regression analysis to be inversely related to yolk sac PCDD/PCDF concentrations. In contrast, the activities of ALAT and ASPAT did not appear to be influenced by *in ovo* exposure of herring gull embryos to organochlorine contaminants. Overall, these data suggest that current organochlorine contamination may be affecting the HPA axis and associated intermediary metabolic pathways in environmentally exposed herring gull embryos in the Great Lakes.

Plasma Corticosterone

Basal plasma corticosterone concentrations of individual herring gull embryos correlated negatively with calculated TEQs and all major classes of organochlorine contaminants except CHCs. This finding is interesting in light of previous studies in which metabolites of the predominant environmental CHC,

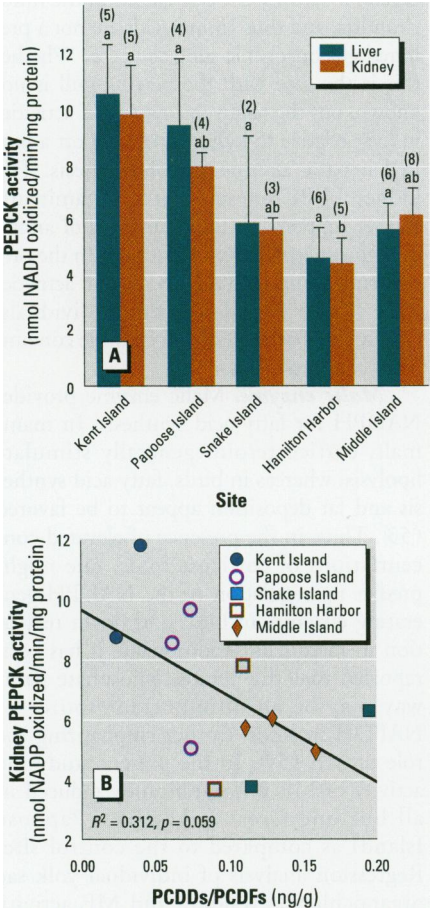

Figure 3. Phosphoenolpyruvate carboxykinase (PEPCK) activity of herring gull embryo liver and kidney cytosol by site (A) and by yolk sac concentrations of PCDDs/PCDFs (B). In (A), bars for each response identified with the same letter among sites are not significantly different from each other (Tukey test, $p < 0.05$; n is shown in parentheses); error bars indicate standard error. In (B), linear regression of individual kidney PEPCK activity data and individual yolk sac polyclorinated dibenzo-*p*-dioxin/polychlorinated dibenzofuran (PCDD/PCDF) concentrations. NADH, reduced nicotinamide adenine dinucleotide.

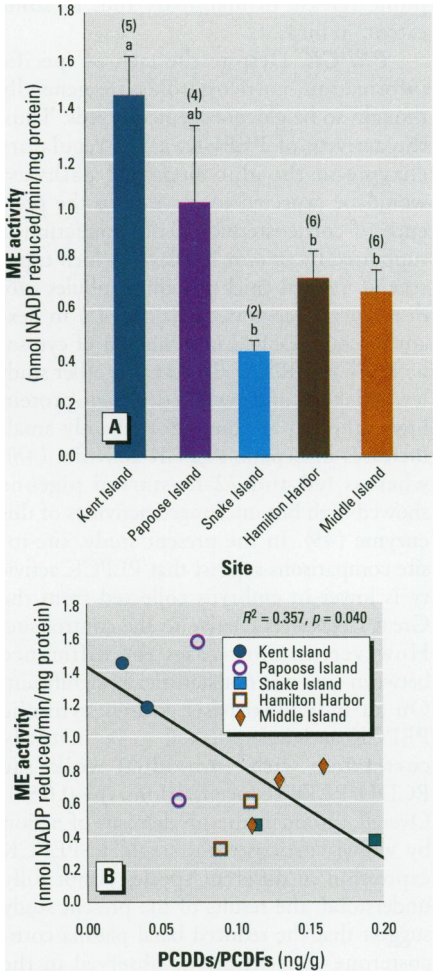

Figure 4. Malic enzyme (ME) activity of herring gull embryo liver cytosol by siteby site (A) and by yolk sac concentrations of PCDDs/PCDFs (B). In (A), bars identified with the same letter among sites are not significantly different from each other (Tukey test, $p < 0.05$; n is shown in parentheses); error bars indicate standard error. In (B), linear regression of individual ME activity data and individual yolk sac polychlorinated dibenzo-*p*-dioxin/polychlorinated dibenzofuran (PCDD/PCDF) concentrations. NADP, nicotinamide adenine dinucleotide phosphate.

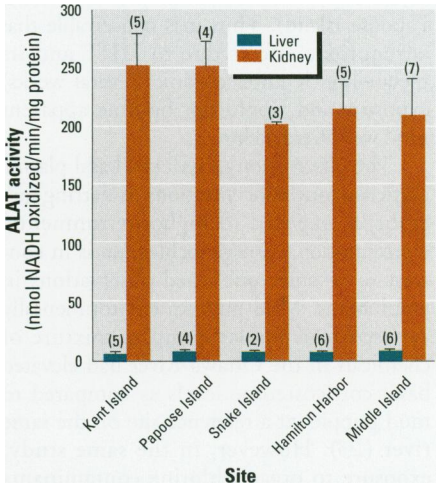

Figure 5. Alanine aminotransferase (ALAT) activity of herring gull embryo liver and kidney cytosol by site. There are no significant differences among sites (Tukey test, $p > 0.05$; n is shown in parentheses); error bars indicate standard error. NADH, reduced nicotinamide adenine dinucleotide.

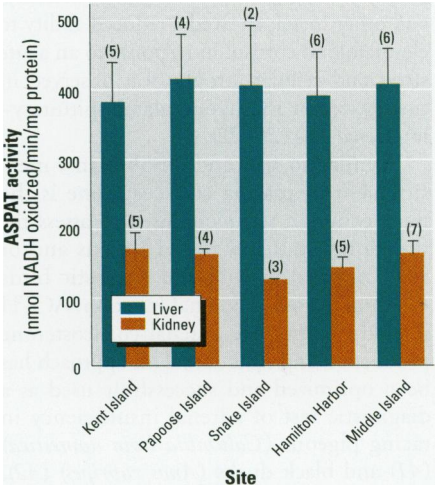

Figure 6. Aspartate aminotransferase (ASPAT) activity of herring gull embryo liver and kidney cytosol by site. There are no significant differences among sites (Tukey test, $p > 0.05$; n is shown in parentheses); error bars indicate standard error). NADH, reduced nicotinamide adenine dinucleotide.

DDT, were shown in laboratory studies to be adrenotoxic agents in birds (*34*). Another study showed significant decreases in adrenal and plasma corticosterone concentrations in 5-week-old chickens treated with as little as 5 ppm *p,p'*-DDT over the course of several weeks (*35*). The primary DDT metabolites measured in brain, adrenal, and liver tissue of chickens during this feeding study were *p,p'*-DDE and *p,p'*-DDD (0.5–2.7 ppm depending on the tissue and metabolite). In comparison, the concentration of the predominant DDT metabolite, *p,p'*-DDE, measured in the herring gull embryo yolk sacs in the present study ranged from as low as 0.44 ppm (individual yolk sac from Kent Island) to as high as 9.75 ppm (individual yolk sac from Papoose Island). Thus it is conceivable that adrenotoxic effects due to DDT and its metabolites requires chronic (several weeks) exposure and would not become apparent until well after hatching.

The observation of reduced basal plasma corticosterone concentrations in herring gull embryos exposed to high environmental concentrations of organochlorines is in contrast to recently published observations in amphibians. Mud puppies environmentally exposed to a similar complex mixture of chemicals in the Ottawa River had elevated basal corticosterone levels as compared to mud puppies at a reference site on the same river (*23*). However, in the same study, exposure to organochlorine contaminants reduced the abilities of the mud puppies to elevate blood corticosterone concentrations in response to an acute stress or ACTH challenge. Similarly, teleost fish environmentally exposed to complex mixtures of bleached kraft pulp mill effluent, PCBs, polycyclic aromatic hydrocarbons, mercury, and other metals showed a reduced ability to elevate blood cortisol in response to an acute stress and cellular atrophy was observed in tissues within the hypothalamo–pituitary–interrenal axis (*20–22*).

Similar to fish and amphibians, measurement of plasma corticosterone is the most common technique used to assess the functional status of the HPA axis and/or adrenal gland in wild and domestic birds (*36–40*). To assess adrenal function, ACTH is used to stimulate adrenal corticosterone production and secretion. This approach has been optimized and successfully used as a diagnostic test of adrenal insufficiency in racing pigeons (*Columbia livia domestica*) (*41*) and black ducks (*Anas rubripes*) (*42*). Stressors, such as heat or cold exposure, effectively stimulate the entire HPA axis, also resulting in adrenal corticosterone synthesis and secretion. Several studies have shown that the HPA axis of the developing chicken embryo is sensitive to changes in environmental temperatures (*43–45*). Furthermore, ACTH administration has been successfully used to induce corticosterone synthesis and secretion in avian embryos (*25,26*). Although experiments were conducted to determine whether adrenal function (using ACTH stimulation) and HPA axis function (using heat exposure) were altered in environmentally exposed herring gull embryos in the present study (data not shown), the sample sizes were insufficient to draw any significant conclusions.

Intermediary Metabolic Enzymes

This study also examined the impact of sampling site and contaminant levels on a number of intermediary metabolic enzymes known to be affected by HPA axis activation and subsequent adrenal steroid hormone release in mammals and, to some extent, in birds.

PEPCK. Despite the lack of specific information, corticosteroids are generally thought to be gluconeogenic in birds. Thus, the activity of PEPCK, a key regulatory enzyme in the gluconeogenic pathway, would be expected to increase in the presence of corticosteroids. This prediction is supported by *in vitro* studies in which exposure of isolated chicken kidney tubules (*46*) or an avian hepatoma cell line (*47*) to dexamethasone resulted in induction of cytosolic PEPCK mRNA. However, in other studies, chickens and owls fed a high-protein low-carbohydrate diet showed only small increases in hepatic PEPCK activities (*48*), whereas fed and 72-hr-starved pigeons showed high but unchanged activities of this enzyme (*49*). In the present study, site-to-site comparisons suggest that PEPCK activity is lower in embryos collected from the Great Lakes sites relative to the control site. However, in most cases the difference between sites was not statistically significant. On an individual basis, kidney cytosolic PEPCK activity appeared to be inversely correlated with individual yolk sac PCDD/PCDF concentrations ($p = 0.059$). Overall, although the mechanism of action by which corticosteroids regulate PEPCK expression in different species is not fully understood, the results of the present study suggest that the reduced basal plasma corticosterone concentrations observed in the highly contaminated embryos may be sufficient to impair regulation of PEPCK activity in these birds.

ASPAT and ALAT. Gluconeogenesis produces glucose from noncarbohydrate precursors, especially amino acids. ALAT and ASPAT are necessary to provide carbon skeletons from alanine and asparate to gluconeogenesis, such that under gluconeogenic conditions and possibly corticosteroid regulation, activities of these two enzymes are increased. Activities of these enzymes may also be elevated because of their role in shuttling malate across mitochondrial membranes for utilization by cytosolic PEPCK—the form that is corticosteroid inducible in all animals studied to date [reviewed by Watford (*46*)]. However, based on previous studies, some caution must be taken when considering avian systems. Whereas avian embryo liver slices can incorporate ^{14}C amino acids into glucose (*50,51*), it was also reported that fasted adult chickens cannot utilize aspartate to produce glucose (*52*). Furthermore, in another study, both chickens and owls fed a low-protein high-carbohydrate diet showed a reduced level of ALAT (*48*). These differences may relate to the species-specific cellular localization of PEPCK. For example, in pigeon liver, PEPCK is localized in the mitochondria, and thus amino acids are not a preferred gluconeogenic substrate (*49*). Whether this is the case with the herring gull is not known, but the relatively low ALAT activities in liver relative to kidney does support a limited hepatic alanine gluconeogenesis. The kidney PEPCK response to contaminants further supports an important role of amino acid gluconeogenesis in this tissue. In the present study, both ASPAT and ALAT activities were similar for all sites and individuals, despite differences in organochlorine contaminant concentrations.

Malic enzyme. Malic enzyme provides NADPH for fatty acid synthesis. In mammals, corticosteroids generally stimulate lipolysis, whereas in birds, fatty acid synthesis and fat deposition appear to be favored (*53*). Thus, in the presence of elevated concentrations of corticosteroids, one might predict up regulation of the NADPH-generating enzymes in birds and down regulation in mammals. Additionally, it has been reported that the pentose phosphate pathway may be an unimportant source of NAPDH in birds, further emphasizing the role of ME (*54*). In the present study the activity of ME was significantly reduced in all but one Great Lakes site (Papoose Island) as compared to the control site. Regression analysis of individual yolk sac organochlorine residues and ME activity revealed a significant inverse relationship between PCDD/PCDF concentrations and ME activity. Based on these findings, it is possible that organochlorine contaminants, particularly dioxins and furans, may have an impact on fatty acid synthesis in herring gull embryos.

CYP1A Induction

Induction of CYP1A has commonly been used as a biomarker of exposure of birds to environmental contaminants, particularly

PCDDs/PCDFs (*55–58*). Because many organochlorine compounds are ligands for the aryl hydrocarbon (Ah) receptor, this end point was included in the original study design. However, despite efforts to optimize the ethoxyresorufin-*O*-deethylase (EROD) assay for herring gull embryo liver microsomes, the activities were approximately 100× lower than those previously reported for herring gull embryo liver fractions prepared by low-speed centrifugation (*55*). Because the formulation of the homogenization buffer used was optimized for measurement of cytosolic intermediary metabolic enzyme activities, and not for the measurement of microsomal EROD activities, it is possible that use of this buffer resulted in lower than expected absolute EROD activities. Thus, until further tissue is collected and analyzed, it is unknown whether CYP1A induction is correlated with any of the end points measured in the present study.

Conclusions

In summary, basal corticosterone concentrations of individual herring gull embryos were found to correlate negatively with individual yolk sac PCDDs/PCDFs, total PCBs, non-*ortho* PCBs, and calculated TEQs. Furthermore, the activities of two intermediary metabolic enzymes—PEPCK and ME—appear to be influenced by dioxins and furans deposited in the yolk sac. The observation of reduced kidney cytosolic PEPCK and hepatic ME activities in individuals exposed to high yolk sac concentrations of PCDDs and PCDFs is consistent with the finding that basal corticosterone concentrations were also reduced in similarly exposed individuals. Hence, reduced basal corticosterone production may result in insufficient induction of key intermediary metabolic enzymes. Although the long-term consequences of down regulation of intermediary metabolic enzymes were not investigated in the present study, it is possible that deficits in gluconeogenic or lipogenic activity may play roles in the wasting observed in young chicks in highly contaminated areas within the Great Lakes basin (*13,59*). Wasting, primarily characterized by loss of body weight and a voluntary decrease in food intake, is a well documented symptom of halogenated aromatic hydrocarbon, and in particular TCDD, toxicity in several species of laboratory animals [reviewed by Poland and Knutson (*60*)]. Furthermore, TCDD inhibits several key gluconeogenic enzymes, including PEPCK, in rats at doses that are in the same range as those causing TCDD-induced appetite suppression and weight loss (*61–63*).

Overall, the data obtained in the present study provide the first evidence that current levels of organochlorine contamination may be affecting the HPA axis and associated intermediary metabolic pathways in environmentally exposed herring gull embryos in the Great Lakes. Additional studies are required to determine whether the functions of the adrenal gland or HPA axis are compromised in embryos exposed to organochlorine contaminants and whether other species, or life stages, of birds show relationships between contaminant residues and HPA axis-related end points similar to those demonstrated here. Furthermore, controlled egg-injection laboratory studies are required to obtain concentration–response data for corticosteroid agonists and antagonists to develop a better understanding of corticosteroid-mediated regulation of intermediary metabolism and the potential influences of environmental contaminants in avian embryos.

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